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Keywords

major histocompatibility complex, class II DNA probe, restriction fragment, length polymorphism

Disciplines

Agriculture | Genetics | Poultry or Avian Science

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IMMUNOLOGY

Analysis of *B-G* and Immune Response Genes in the Iowa State University S1 Chicken Line by Hybridization of Sperm Deoxyribonucleic Acid with a Major Histocompatibility Complex Class II Probe[1]

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ABSTRACT Sperm DNA was isolated from chickens of the Iowa State University S1 line. Birds were from sublines selected for B-G antigen, humoral immune response to glutamic acid-alanine-tyrosine (IrGAT), and response to Rous sarcoma virus-induced (RSV) tumors. The DNA was digested with restriction enzymes and subjected to Southern blot analysis with a DNA probe specific for a class II gene of the chicken major histocompatibility complex (MHC).

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(*Key words:* major histocompatibility complex, class II DNA probe, restriction fragment length polymorphism)

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INTRODUCTION

The *B* complex was first described by Briles *et al.* (1950) as a blood group locus. Schierman and Nordskog (1961) showed a correlation between *B* blood group differences and skin graft rejection, thus demonstrating that the *B* blood group locus is a marker for the chicken major histocompatibility complex (MHC). Genes in the chicken MHC encode three groups of antigens designated B-L, B-F, and B-G (Pink *et al.*, 1977). The B-L antigens (homologous to class II MHC antigens of mammals) are expressed on B cells and stimulated T cells; the B-F antigens (homologous to class I MHC antigens of mammals) are expressed on almost all cell types (Ewert *et al.*, 1984; Crone *et al.*, 1985). The B-L antigens consist of one α and one β chain that are bound to the cell surface (Guillemot *et al.*, 1986) and are involved in regulation of immune cell cooperation (Vainio *et al.*, 1984). The B-G antigens (designated as class IV), of which no mammalian homolog has been identified, are expressed on erythrocytes only (Longenecker and Mosmann, 1981).

Associations have been demonstrated, in the Iowa State University (ISU) S1 chicken line, between B blood group type and mortality and egg production (Nordskog *et al.*, 1973, 1977), immune response to several antigens (Pevzner *et al.*, 1975, 1979), juvenile body weight (Kim *et al.*, 1987), and resistance to Marek's disease (Steadham *et al.*, 1987) and to fowl cholera (Lamont *et al.*, 1987a). Identification of the MHC in the previous studies was done serologically with anti-B-G antisera. The purpose of the present research was to study the S1 chicken line by analysis of restriction patterns generated by hybridization of sperm DNA with an MHC class II probe.

MATERIALS AND METHODS

Animals. Chickens used were from the ISU S1 White Leghorn line, produced and maintained at the ISU Poultry Science Research Center. Birds were produced from eight sublines, selected for B blood type (B^1B^1 or $B^{19}B^{19}$) humoral immune response to glutamic acid-alanine-tyrosine (IrGAT-high or IrGAT-low), and family response to Rous sarcoma virus-induced (RSV) tumors (regression or progression) (Cheng, 1985; Lamont *et al.*, 1987a). Birds heterozygous for B blood type, IrGAT, and RSV response were also used. Birds were blood typed with B-G specific antisera by the microhemagglutination method (Wegmann and

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Smithies, 1966). The IrGAT phenotype was determined by radioimmunoassay (Pevzner *et al.*, 1978). The RSV tumor response type was assigned to families based on tumor response of sibs (Gebriel *et al.*, 1979). Sexually mature males were used as the source of semen.

Deoxyribonucleic Acid Isolation. The DNA was isolated from semen. Fifty μL of semen were diluted in 1 mL of phosphate buffered saline-EDTA (.14 *M* NaCl, .01 *M* Na_3PO_4, 1 m*M* EDTA, pH 7.0) to which 10 mL of lysis buffer [10 m*M* tris(hydroxymethyl)aminomethane, pH 8.0, 100 mM NaCl, 10 mM EDTA, .5% sodium dodecyl sulfate (SDS), 2% 2-mercaptoethanol] and 2 mg RNaseA were added. The mixture was incubated for 30 min at room temperature. Five milligrams of proteinase K were added and incubated at 50 C for 2 h and then at 37 C overnight. The next day, one phenol extraction, three extractions with phenol:chloroform:isoamyl alcohol (23:23:1), and two extractions with chloroform:isoamyl alcohol (23:1) were performed. After the last extraction, the DNA was precipitated in 2.5 vol. of 95% ethanol (–20 C) and .1 vol. of 2 *M* NaAc (pH 5.2) at –20 C overnight. The pellet was air-dried for 30 min and then dissolved in 2 mL H_2O. The DNA concentration and purity was determined by the ratio of absorbance at 260 nm and 280 nm.

Restriction Enzyme Digestion. Ten micrograms of DNA were digested with 60 to 100 units of enzyme in buffer (as recommended by the manufacturer) and 10 m*M* spermidine and then incubated for 2 h at 37 C. Enzymes used are listed in Table 1.

Electrophoresis and Transfer of DNA Fragments. Fragments of DNA were separated on .8% agarose gels in tris borate electrophoresis buffer. Ethidium bromide (20 μg) was added to the gel and the buffer. Wells of the gel were loaded with 20 μL of digested DNA. HindIII-cut lambda was used as a fragment size standard. Electrophoresis was carried out at 30 mA for 16 to 20 h. Transfer of DNA fragments was carried out for 18 h in 20× standard sodium citrate (SSC) according to the procedure of Southern (1975).

Probe. A chicken class II genomic probe, kindly provided by C. Auffray, Centre National de la Recherche Scientifique, Nogent-sur-Marne, France, was used. This probe is part of the β_2 exon of class II and is 234 base pairs in length (Bourlet *et al.*, 1988). The probe was labeled by nick translation (Maniatis *et al.*, 1982).

Prehybridization and Hybridization. Filters were prehybridized for 6 h at 42 C in a shaking

TABLE 1. *Associations of restriction fragment length polymorphisms identified by a major histocompatibility complex class II deoxyribonucleic acid probe with traits in the Iowa State University S1 chicken line*

Restriction enzyme	B blood group	IrGAT[1]	RSV response[2]
PvuII	+[3]	–[4]	–
BglII	+	–	ND[5]
Sau3A	+	–	ND
EcoRI	–	–	–
HindIII	–	–	–
BamHI	–	–	–
TaqI	–	–	ND
BclI	–	–	ND
HpaII	–	–	ND
KpnI	–	–	ND
HhaI	ND	–	ND
SstI	ND	–	ND
EcoRV	ND	–	ND
XhoI	ND	–	ND
PstI	ND	–	ND

[1] IrGAT = Humoral immune response to glutamic acid-alanine-tyrosine.

[2] Response to Rous sarcoma virus-induced tumors.

[3] Restriction fragment length polymorphisms (RFLP) were associated with this trait.

[4] No RFLP were associated with this trait.

[5] ND = Not determined.

water bath in a solution containing 45% 12× SSC in .1 *M* Na_3PO_4, 8 m*M* EDTA (pH 7.0), 45% deionized formamide, and 10% of the following solution: a) 6× SSC in 100 m*M* Na_3PO_4, 8 m*M* EDTA (pH 7.0); b) 2% polyvinylpyrolidone; c) 2% Ficoll 400, d) 10% SDS. Denatured (100 C for 5 min) salmon sperm (100 μg/mL) was added. After prehybridization, filters were rinsed with prehybridization solution without salmon sperm. Hybridization solution (50% 12× SSC, 50% deionized formamide) was added. Air bubbles were pushed out of the hybridization bag, and .1 g probe with 1-3 × 10^7 counts per minute per filter was added. Hybridization was carried out at 42 C in a shaking water bath for 18 h. After hybridization, the filters were washed four times at room temperature in a solution containing 2× SSC, .1% SDS and then two times for 15 min at 58 C in a solution containing .1× SSC, .1% SDS. Filters were air dried for 30 to 60 min, wrapped, and exposed in a cassette with Kodak XAR-5 X-ray film and lightening plus intensifying screens at –70 C for 24 to 48 h.

RESULTS

Patterns of DNA fragments resulting after cleavage with a particular restriction enzyme, electrophoresis, transfer to a hybridization membrane, and probing with a DNA class II MHC probe (Southern blots) are summarized in Table 1. Only three (PvuII, BglII, Sau3A) of the 15 restriction enzymes tested showed restriction fragment length polymorphisms (RFLP) associated with traits of selection in the S1 line. An example of the pattern given by one of the enzymes (PvuII) that showed RFLP is given in Figure 1, and an example of one of the enzymes that did not show any polymorphism (EcoRI) is given in Figure 2. The extra band [6.0 kilobases] in Lane 6 of Figure 2 appeared in 1 of 22 chickens tested. A summary of RFLP generated by PvuII, Sau3A, BglII, and EcoRI is given in Table 2.

In order to test the Mendelian inheritance of the observed RFLP, two F_1 birds were crossed and their offspring analyzed. Figure 3 shows the restriction pattern generated after PvuII digestion of DNA samples from heterozygous B^1B^{19} birds. The DNA of heterozygous B^1B^{19} birds yields a pattern of four bands, including the two bands common to homozygous B^1B^1 and $B^{19}B^{19}$ birds, plus each of the unique bands seen in B^1B^1 or $B^{19}B^{19}$ samples.

DISCUSSION

This study used an MHC class II genomic probe of chicken origin to demonstrate genomic variation among chickens differing for serologically detected class IV antigens. Digestion of DNA with PvuII, BglII, or Sau3A, three of the fifteen enzymes used, generated RFLP that were

TABLE 2. *Size (kilobases) of three major histocompatibility class II deoxyribonucleic acid fragments generated by restriction endonuclease digestion by four enzymes of genomic deoxyribonucleic acid of Iowa State University S1 chickens*

PvuII			Sau3A		BglII		EcoRI	
1,1	19,19	1,19	1,1	19,19	1,1	19,19	1,1	19,19
(kilobase)								
					>23	>23		
						23	23	23
							9.4	9.4
					6.7	6.7		
	5.0	5.0						
4.3	4.3	4.3						
			3.8					
			3.3	3.3				
3.1		3.1						
			2.2					
			2.1					
			.9					
.7	.7	.7	.7	.7				
			.6	.6				

associated with the B-G antigen of the birds. The restriction enzyme PvuII has also been effective with other genetic lines of chickens and with mammalian MHC probes in generating RFLP associated with B blood types (Andersson *et al.*, 1987; Lamont *et al.*, 1987b). Analysis of DNA from heterozygous B^1B^{19} chickens yielded the combined pattern of both homozygous types, suggesting a classical Mendelian pattern of inheritance of class II MHC RFLP patterns associated with the class IV antigens of these birds.

With the use of certain restriction enzymes, there were minor variations in patterns within a B-G type of the S1 line. An example is shown in Figure 2 (Lane 6). Of a total of 22 individual birds analyzed with EcoRI and 12 birds analyzed with BglII, only 1 and 2, respectively, did not conform to the common pattern for that enzyme and B blood type. Because the S1 line is about 40% inbred (Cheng, 1985), these variations may reflect true differences among individual birds. The variations were probably not due to technical difficulties, because DNA was isolated from the individuals on two independent occasions and identical extra bands were detected upon each analysis.

Use of the class II MHC probe did not generate RFLP associated with IrGAT. Previous work by Benedict *et al.* (1975) showed an association between the chicken MHC and IrGAT. Studies with the S1 line demonstrated a recombination between genes encoding IrGAT and serologically determined erythrocyte antigens (Pevzner *et al.*, 1978), which was the basis for the generation of the sublines analyzed in the research study. There are several possible explanations for the unexpected observation that a class II MHC probe did not produce RFLP that correlated with IrGAT. First, the choice of

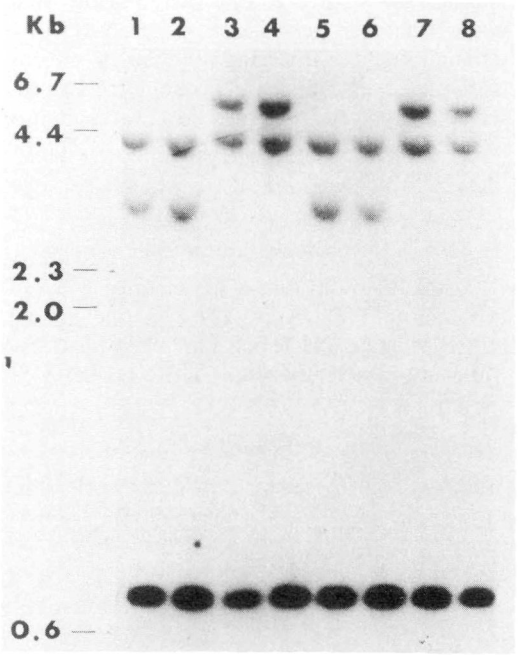

FIGURE 1. Autoradiogram of PvuII-digested fragments of sperm DNA hybridized with a chicken class II major histocompatibility probe. Fragment lengths (from HindIII-cut λ) are designated in the left margin; Kb = kilobase. The B-G antigen type, immune response to glutamic acid-alanine-tyrosine (IrGAT), and Rous Sarcoma virus response are: 1) B^1B^1, IrGAT-low, progressor; 2) B^1B^1, IrGAT-low, regressor; 3) $B^{19}B^{19}$, IrGAT-high, progressor; 4) $B^{19}B^{19}$, IrGAT-low, regressor; 5) B^1B^1, IrGAT-high, regressor; 6) B^1B^1, IrGAT-high, progressor; 7) $B^{19}B^{19}$, IrGAT-high, regressor; 8) $B^{19}B^{19}$, IrGAT-low, progressor.

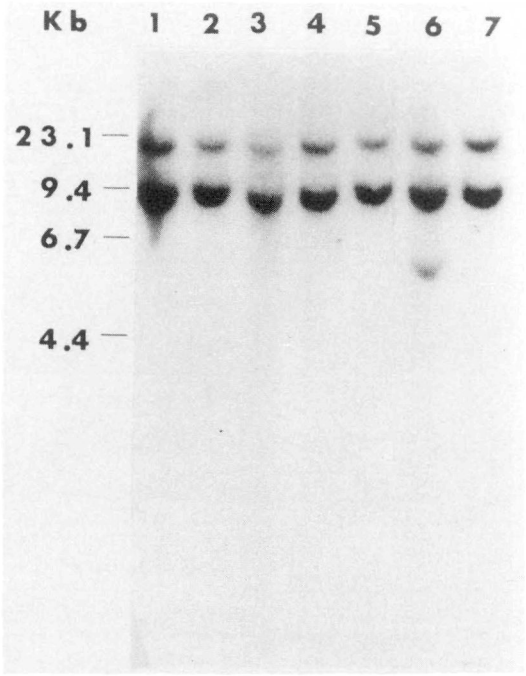

FIGURE 2. Autoradiogram of EcoRI-digested fragments of sperm DNA hybridized with a chicken class II MHC probe. Fragment lengths (from HindIII-cut λ) are designated in the left margin; Kb = kilobase. The B-G antigen type, immune response to glutamic acid-alanine-tyrosine (IrGAT), and Rous Sarcoma virus response are: 1) B^1B^1, IrGAT-low, regressor; 2) $B^{19}B^{19}$, IrGAT-high, progressor; 3) $B^{19}B^{19}$, IrGAT-low, regressor; 4) B^1B^1, IrGAT-high, regressor; 5) $B^{19}B^{19}$, IrGAT-low, progressor; 6) B^1B^1, IrGAT-high, progressor; 7) $B^{19}B^{19}$, IrGAT, high, regressor.

restriction enzymes may not have included any that identify restriction sites differing between birds that differ for IrGAT. Many restriction enzymes were tested (Table 1) but none of them revealed any polymorphisms associated with IrGAT. Second, the immune response to GAT may be encoded outside the MHC. Third, the control of IrGAT may be by one of the α chain class II genes, which are thought to be undetectable by the probe that was used.

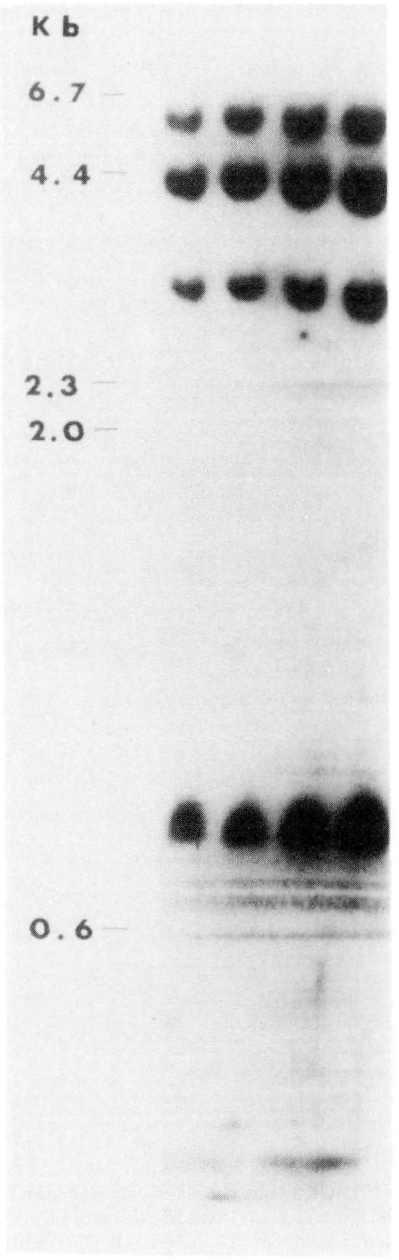

FIGURE 3. Autoradiogram of PvuII-digested fragments of sperm DNA from four B^1B^{19} chickens, hybridized with a chicken class II major histocompatibility complex probe. Fragment lengths (from HindIII-cut λ) are designated in the left margin; Kb = kilobase.

The RSV response of the S1 chicken line was not associated with RFLP identified by a class II MHC probe. A gene exerting major control over RSV tumor regression has been mapped to the MHC class I region (Pláchy and Vilhelmová, 1984), but non-MHC genetic control of RSV response has also been demonstrated (Collins and Zsigray, 1984; Gilmour *et al.*, 1986). Because selection for RSV tumor response in the S1 line was conducted within each MHC haplotype (as determined by B-G antigen and IrGAT phenotype), non-MHC genes probably determined the divergence in RSV response (Kim *et al.*, 1987). It was not, therefore, expected that MHC-probed RFLP would be associated with RSV response in the ISU S1 line of chickens.

In summary, RFLP can be generated with appropriate restriction enzymes and an MHC class II chicken genomic probe used to distinguish individuals differing for class IV serologically detected antigens. This demonstrates that RFLP analysis of DNA may be a useful addition to serological identification of MHC haplotypes in chickens.

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